

BIOGRAPHICAL SKETCH

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NAME: Stroud, Robert M.

eRA COMMONS USER NAME (credential, e.g., agency login): stroud

POSITION TITLE: Professor of Biochemistry and Biophysics, and Professor of Pharmaceutical Chemistry

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Cambridge University, UK: Clare Coll.	BA (Cantab)	May 1964	Natural Science/Chem, Phys
Cambridge University, UK: Clare Coll.	MA (Cantab)	May 1968	Natural Science/Physics
London University, UK: Birkbeck Coll.	MS., PhD.,	Sept 1968	Biological Crystallography
California Institute of Technology, USA	Postdoctoral R.E.Dickerson	July 1971	Crystal structure of Trypsin.

A. Personal Statement

One of my main interests is to determine the atomic resolution structures of the multiple states in the functional cycle of target transmembrane transporters. This includes X-ray crystallography, electron cryo microscopy, chemical cross-linking, and other methods, and thus also requires integrative structure modeling to compute models consistent with all available information, regardless of its source. I am particularly excited about the possibility to determine the free energy differences between the states, and even the kinetics of their interconversions, based in part on an analysis of single particle electron microscopy images and molecular dynamics simulations.

Specifically in the area of this proposal we determined the structure of a close bacterial homolog of the mammalian Vesicular Glutamate (VGLUT) transporters, *E. coli* D-galactonate/H⁺ symporter DgoT in two states: one open to the cytoplasmic side, and the other open to the periplasmic side with substrate¹. The different structures that identify a proton translocation pathway conserved from bacteria to mammals. Functional analysis suggests that a transition in the role of H⁺ from flux coupling to allostery underlies the divergence in energy source between the bacterial homolog that works like sialin, and the VGLUTs driven by potential.

We now seek to harness Electron cryomicroscopy (CryoEM). Using CryoEM at the record resolution for an ATP-driven ABC transporter, we opened the way to understanding the multiple state mechanism, in *Nature*². We also developed the use of Fab fragments to enlarge the structures of DgoT, and other MFS transporters for cryoEM³. Second we seek to determine structures of eukaryotic Vesicular glutamate transporters of ~ 65kDa. In size. In the first eukaryotic structure of an intracellular Calcium pump VCX, in the Calcium/cation family, we saw by comparison with a prokaryotic NCX proton gradient the large conformation changes that links H⁺ movement inward, to Ca⁺⁺ outward via different, structurally coupled paths, in *Nature*. Through our atomic structure of the first eukaryotic 'MFS' family transporter⁴, we show that the proton and substrate paths coupled intricately through conformation change in the transporter in *Nature*⁴. We report the first drug-bound structures of the human glucose transporter GLUT1. These latter structures are of eukaryotic transporters that are, like the SLC17 family that we propose to study, in the Major Facilitator Superfamily (MFS family).

In previous years I determined structures of many aquaporins, the first structure and mechanism of any ammonia channel (Rh family) in 2004 and its regulation- and the human RhCG, and RhAG and their mechanisms at atomic resolution. In 1999 we determined the Erythropoietin (EPO)-EPO receptor complex (second structure of any cytokine-receptor 1:2 complex close after hGH). In 1979-1990 we determined the low dose electron microscopic profile structures of the acetylcholine receptor showing the large extracellular vestibule surrounded by five similar transmembrane subunits, the atomic structure of α -bungarotoxin and how it acts. We determined the channel forming colicin Ia and Bacillus thuringiensis toxins that illustrate membrane channel formation. We provided >45 PDB depositions of helical transmembrane protein structures.

Traffic: Our structures of the membrane-targeting 'signal recognition particle' in complex with its receptor showed how these targeting GTPases reciprocally activate each other, and 'hand hold' integral membrane and secreted proteins to the transmembrane protein-pore, the translocon. We also determined the structure of a

transmembrane translocon in its opened, - translocating-like state. Our structures of the Unfolded Protein Response element, Ire1, show how unfolded proteins are detected in the ER and relay signals to the cytoplasm.

Enzymes & Drug Discovery: I derived mechanisms and structures of paradigmatic proteins and their complexes. This began with my own first X-ray crystal structure of trypsin, trypsinogen in 1970 and the mechanisms of proteases. We deposited >300 PDB atomic structures of protein complexes, protein-RNA complexes, the first phosphoregulation mechanism, for isocitrate dehydrogenase, structure assisted drug design aimed at therapeutic drug target proteins particularly HIV protease, thymidylate synthase, serine proteases, two-domain HIV integrase. We produced the most detailed stereochemical mechanism for Thymidylate synthase, HIV-protease, and for many drug complexes of these critical drug target enzymes. We determined the structural basis of RNA recognition, methylation and pseudouridylation. (3 patents)

1. Leano JB, Batarni S, Eriksen J, Juge N, Pak JE, Kimura-Someya T, Robles-Colmenares Y, Moriyama Y, **Stroud RM*** and Edwards RE* (2018) *Structural Basis for Energy Coupling by a Family of Organic Anion Transporters* Nature Structural & Molecular Biology – in press
2. Kim* J, Wu* S, Tomasiak* TM, Mergel C, Winter MB, Stiller SB, Robles-Colmanares Y, **Stroud RM**, Tampé R, Craik CS, Cheng Y. (2015) *Subnanometre-resolution electron cryomicroscopy structure of a heterodimeric ABC exporter*. Nature. **517**: 396-400. doi: 10.1038/nature13872. (PubMed PMID: 25363761/PMCID: PMC4372080)
3. Wu S, Avila-Sakar A, Kim J, Booth DS, Greenberg CH, Rossi A, Liao M, Li X, Alian A, Griner SL, Juge N, Yu Y, Mergel CM, Chaparro-Riggers J, Strop P, Tampe R, Edwards RH, **Stroud RM**, Craik CS, Cheng Y. (2012) *Fabs enable single particle cryoEM studies of small proteins*. Structure **20**: 582-92. (PMCID: PMC3322386)
4. Pedersen BP, Kumar H, Waight AB, Risenmay AJ, Roe-Zurz Z, Chau BH, Schlessinger A, Bonomi M, Harries W, Sali A, Johri AK, **Stroud RM** (2013) *Crystal structure of a eukaryotic phosphate transporter*. Nature. **496**: 533-6. (PMCID: PMC3678552)

B. Positions and Honors.

Positions and Employment

- | | |
|----------------|--|
| 1973 - 1977 | Instructor (1971-1973) Assistant Professor (1973-75) and Associate Professor of Chemistry, (1975-77) California Institute of Technology, Pasadena, California. |
| 1977 - present | Associate Professor (1977-79), Professor of Biophysics & Biochemistry (1979 - present) Dept. of Biochemistry & Biophysics, and Dept. of Pharmaceutical Chemistry, University of California, San Francisco. |

Honors (selection)

- | | |
|-------------|---|
| 1984 | DeWitt Stetten Lecture, NIGMS |
| 1988 - 1989 | President of the Biophysical Society of USA |
| 1992 - | FRSM Fellow of the Royal Society of Medicine (United Kingdom) |
| 2000 - | Founding class Fellow of the Biophysical Society of US |
| 2003 - | NAS Member of the National Academy of Sciences |
| 2007 - | Fellow of the American Academy of Arts and Sciences |
| 2008 | Hans Neurath Award of the Protein Society US |
| 2009 | Anatrace Award of the Biophysical Society US |
| 2006 - 2016 | NIH Merit Award |
| 2014 | 8 th C.B Anfinsen Memorial Lecture, Weizmann Institute |
| 2014 | Keynote Frontiers in Membrane Protein Structure and Dynamics, University of Chicago |
| 2015 | The Inaugural Lecture, Indian Microbiology Society, New Delhi, India |
| 2017 | The E.W.Hughes Lecture at Caltech (shared with David Eisenberg) |
| 2018 | The Faculty Research Lecturer at UCSF |

Other Experience and Professional Memberships

- | | |
|-------------|--|
| 1993 - 2004 | The Editor, <i>Annual Review of Biophysics and Biomolecular Structure</i> . |
| 1997 - 2009 | Member and Chair (2006-) Scientific Advisory Board, St Jude Children's Cancer Research Hospital. |
| 2007 - 2010 | Chair National Academy of Sciences Biophysics and Computational Biology Section |
| 2004 - 2014 | Director Membrane Protein Expression Center (mpec.ucsf.edu) |
| 2005 - 2015 | Director Center for Structure of Membrane Proteins (csmp.ucsf.edu) |

C. Contribution to Science 'our'/'I' refers to myself and my immediate research group of scholars.

1. Secondary Transporters/Receptors: Our recent study of a homolog of the VGLUTs provided multiple states of this transporter. Our study of phosphate transporters revealed the first structure of any eukaryotic transporter and receptor (tranceptor) of the major facilitator super (MFS) family⁴. Recognizing the importance of human and eukaryotic membrane proteins for therapeutics, our unique expression system in *S.cerevisiae* is tailored to integral membrane proteins and makes it easy to screen stabilizing agents, ligands, lipids, detergents thermodynamically. This structure made it possible to interpret the wealth of mutational data on homologous yeast Pho84. This gave rise to hypothesis as to how it worked and mutational tests of transport in liposomes that we devised. I sought to find ways of seeing transporters in action, and how this is coupled to driving ions or protons. This we achieved with Kaback, with his mutations in LacY, and showed how, and when proton transfer is coupled to alternating access⁷. We also defined multiple states in a sodium driven, calcium ion antiporter VCX⁶. We sought to define the human glucose transporter GLUT1, a cancer target, and show how it is inhibited by cytochalasin, and drug leads, seeking an anti-cancer therapeutic⁸.

5. Pak JE, Ekende EN, Kifle EG, O'Connell JD, 3rd, De Angelis F, Tessema MB, Derfoufi KM, Robles-Colmenares Y, Robbins RA, Goormaghtigh E, Vandenbussche G, **Stroud RM**. (2013) Structures of intermediate transport states of ZneA, a Zn(II)/proton antiporter. *Proc Natl Acad Sci U S A*. 110:18484-18489. PubMed PMID: 24173033; PubMed Central (PMCID: PMC3832018)
6. Waight AB, Pedersen BP, Schlessinger A, Bonomi M, Chau BH, Roe-Zurz Z, Risenmay AJ, Sali A, **Stroud RM** (2013) Structural basis for alternating access of a eukaryotic calcium/proton exchanger. *Nature*. **499**: 107-10. (PMCID: PMC3702627)
7. Kumar H, Finer-Moore JS, Kaback HR, **Stroud RM**. (2015) Structure of LacY with an alpha-substituted galactoside: Connecting the binding site to the protonation site. *Proc Natl Acad Sci U S A*.;112(29):9004-9. doi: 10.1073/pnas.1509854112. (PMID: 26157133/PMCID: PMC4517220)
8. Kapoor K, Finer-Moore J, Pedersen BP, Waight, Caboni L, Hillig R, Bringmann P, Heisler I, Mülle T, Siebeneicher H, **Stroud RM** (2016) Mechanism of inhibition of human glucose transporter GLUT1 is conserved between cytochalasin and phenylalanine amides. *Proc Natl Acad Sci U S A*. on line Apr 12 2016 DOI 10.1073/pnas.1603735113 (PMID: 27078104/PMCID: PMC4855560)

2. Seeing alternate states by CryoEM: Our first structure of a heterodimeric ABC transporter (150kDa) set a record at 8Å resolution for a molecule of this size in 2015⁹. Ours was the first cryoEM structure of any ABC transporter, and it opens the entire field of ABC transporters revealing multiple states that show how ATP hydrolysis is coupled to transport. I had already purified, and crystallized the ABC transporter TmrAB and determined its X-ray atomic structure to 2.8Å atomic resolution. Our publication outlines a revolutionary new approach to determining integral membrane protein structures, combining cryoEM with our X-ray structure of a single state. We increased the size, and ability to orient samples using Phage displayed Fab fragments. I initiated the overall plan, my group devised protein expression and purification of all the proteins in the project, and I co-wrote the paper with Craik and Cheng.

My engagement with cryoEM goes back to 1981 when with postdoctoral Steven Hayward, I devised the then highest resolution cryoEM, first image-based phasing to 3.7Å resolution, with intensities to 2.65Å for bacteriorhodopsin¹⁰. I followed this with the first application of the three dimensional 'envelope' as a means of phasing continuous diffraction along the lattice lines of electron diffraction in 1979-¹¹. We carried out the first 3-D reconstruction of the Acetylcholine receptor by low dose EM in 1989¹².

9. Kim* J, Wu* S, Tomasiak* TM, Mergel C, Winter MB, Stiller SB, Robles-Colmanares Y, **Stroud RM**, Tampe R, Craik CS, Cheng Y. (2015) Subnanometre-resolution electron cryomicroscopy structure of a heterodimeric ABC exporter. *Nature*. **517**: 396-400. doi: 10.1038/nature13872. (PMID: 25363761/PMCID: PMC4372080)
10. Hayward SB, **Stroud RM**. (1981) Projected Structure of Purple Membrane Determined to 3.7 Å Resolution by Low Temperature Electron Microscopy. *J. Mol. Biol.* **151**, 491-517. (PMID: 7338903)
11. Agard DA, **Stroud RM**. (1982) Linking Regions Between Helices in Bacteriorhodopsin Revealed. *Biophys. J.* **37**, 589-602 (PMID: 7074187)
12. Mitra AK, McCarthy MP, **Stroud RM**. (1989) Three-Dimensional Structure of Nicotinic Acetylcholine Receptor and Location of the Major Associated 43-kD Cytoskeletal Protein, Determined at 22 Å by Low Dose Electron Microscopy and X-Ray Diffraction to 12.5Å. *J. Cell Biol.* **109**, 755-774. (PMID: 2760111)

3. Channels and Receptors: In 2016 our first crystal structure of any eukaryotic intracellular ion channel, an endolysosomal channel that determines trafficking, at 2.8Å resolution, TPC1¹³. It regulates the import of

ligands and viruses into the cell via endolysosomes, and the decisions to trigger degradation, recycling to the plasma membrane, or autophagy. We show how an inhibitor, that cures already Ebola-infected mice acts to gate the channel allosterically. This is the first exogenously expressed, eukaryotic, intracellular voltage and ligand gated ion channel. We also determined the structural basis for protein secretion through the Signal Recognition pathway with structures of the SRP and its receptor SR, and the transmembrane translocon, - the pore through which nascent protein chains are threaded¹⁰. This structure of a protein-channel¹⁴ 'caught in action' provided the last step in our work on Signal Recognition Particle directed targeting of membrane proteins to membrane insertion.

My work on transmembrane channels began in 1971-present and led to the first 3-dimensional structure of the 350kDa. acetylcholine receptor by X-ray and low dose Electron Microscopy¹⁵, and the mechanisms of membrane channel formation and structure by toxins, by colicin Ia, and peptides.

We first defined the mechanism of the EPO receptor from the EPO-EPO receptor complex in 1998¹⁶, - the second cytokine-receptor complex structure that yielded new insights into the mechanisms of cytokine receptor orientation and signaling.

13. Kintzer A, **Stroud RM** (2016) Structure, inhibition and regulation of two-pore channel TPC1 from *Arabidopsis thaliana*. *Nature*. **531**: 258-62 DOI 10.1038/nature17194 (PMCID: PMC4663122)
14. Egea PF, Stroud RM. (2010) Lateral opening of a translocon upon entry of protein suggests the mechanism of insertion into membranes. *Proc Natl Acad Sci USA*. 40, 17182-7, 2010. (PMID: 20855604/PMCID: PMC2951439)
15. Mitra AK, McCarthy MP, **Stroud RM**. (1989) Three-Dimensional Structure of Nicotinic Acetylcholine Receptor and Location of the Major Associated 43-kD Cytoskeletal Protein, Determined at 22 Å by Low Dose Electron Microscopy and X-Ray Diffraction to 12.5Å. *J. Cell Biol.* **109**, 755-774. (PMID: 2760111)
16. Syed RS, Reid SW, Li C, Cheetham JC, Aoki KH, Liu B, Zhan H, Osslund TD, Chirino AJ, Zhang J, Finer-Moore J, Elliot S, Sitney K, Katz BA, Matthews DJ, Wendoloski JJ, Egrie J, **Stroud RM**. (1998). Efficiency of signaling through cytokine receptors depends critically on receptor orientation. *Nature* **395**, 511-516. (PMID: 9774108)

4. **Aquaporins:** In 2000 we determined¹⁷ the first structure of any aquaporin at atomic resolution, a landmark discovery in the field of water transport. Our structure was recognized in Agre's Nobel prize lecture in 2003. It was the first channel for uncharged species because electrical signals from ion channels makes them much easier to detect, to assay, and to test. The structure of a truncated bacterial potassium channel had been described two years earlier in April 1998 by Rod Mackinnon, who shared the Nobel with Agre. Our structure first explained selectivity for water, and the absolute exclusion of any protons or ions. We followed with atomic structures and mechanisms of several more key aquaporins: AqpZ from *E.coli*, human AQP4 important in human brain, stroke, and target in the autoimmune disease NMO¹⁸, bovine eye lens AQP0 revealed mechanisms of the eye lens¹⁹. *Plasmodium* aquaporin also combined a very fast water channel and a very fast glycerol channel, presaged by mutations that allowed us to define the basis for each²⁰.
17. Fu D, Libson A, Miercke L, Weitzman C, Nollert P, Krucinski J, **Stroud R.M.** (2000) The Structure of a Glycerol Conducting Channel Reveals the Basis for its Selectivity. *Science* **290**, 481-486. (PMID: 11039922).
18. Ho JD, Yeh R, Sandstrom A, Chorny I, Harries WE, Robbins RA, Miercke LJ, & **Stroud RM**. (2009) Crystal structure of human aquaporin 4 at 1.8 Å and its mechanism of conductance. *Proc Natl Acad Sci USA* **106**, 7437-7442. (PMID: 19383790/PMCID: PMC2678640)
19. Harries WEC, Akhavan D, Miercke LJW, Khademi S, **Stroud RM**. (2004) *Proc Nat Acad Sci* **101**, 14045-14050. The Channel Architecture of Aquaporin 0 At 2.2 Å Resolution. (PMID: 15377788)
20. Newby ZE, O'Connell J, 3rd, Robles-Colmenares Y, Khademi S, Miercke LJ, **Stroud RM**. (2008) Crystal structure of the aquaglyceroporin PfAQP from the malarial parasite *Plasmodium falciparum*. *Nature Struct Mol Biol* **15**, 619. . (PMID: 18500352/PMCID: PMC2568999)

5. **Ammonia Transporters:** In 2004 ours²¹ was the first structure and mechanism of any member of the ammonia transporter family, the Rh factor family in mammals, at resolution of 1.35Å. It was recognized as one of the 4 discoveries of the year in Chemistry of C&E News. Our structure showed that this 11-crossing integral membrane protein was a channel for uncharged NH₃ rather than a transporter, resolving a conundrum in the entire field, verified by pH change in liposomes. The paper was a landmark defining the structural landscape for the family. We followed with publication of the complexes formed with regulatory GlnK that sense the energy health of the cell²², both in terms of the ATP/ADP ratio, and in carbon health by

the glutamate to glutamine ratio and revealed the mechanisms of regulation. We followed with structures of human Rh factors RhCG²³, and RhAG. These papers first defined the chemical and structural basis of the ammonia transport field.

21. Khademi S, O'Connell III J, Remis J, Robles-Colmenares Y, Miercke LJW, **Stroud RM**. (2004) Mechanism of ammonia transport by Amt/MEP/Rh: structure of AmtB at 1.35 Å [Cover Article, with Perspective by Knepper, Agre. p1573-1574]. *Science* **305**, 1587-1594. (PMID: 15361618).
22. Gruswitz F, O'Connell J, 3rd, **Stroud RM**. (2007) Inhibitory complex of the transmembrane ammonia channel, AmtB, and the cytosolic regulatory protein, GlnK, at 1.96 Å. *Proc Natl Acad Sci USA* **104**, 42-47. (PMCID: PMC1765473).
23. Gruswitz F, Chaudhary S, Ho JD, Schlessinger A, Pezeshki B, Ho C-M, Sali A, Westhoff C.M, **Stroud, RM**. (2010) Function of human Rh based on structure of RhCG at 2.1 Å. *Proc Natl Acad Sci USA*. **107**, 9638-43. (PMCID: PMC2906887)

Complete List of Published Work in MyBibliography: 322 publications

<http://www.ncbi.nlm.nih.gov/sites/myncbi/1NSIVtL66fK/bibliography/40382941/public/?sort=date&direction=descending>

D. Research Support.

Ongoing Research Support

R01 NS089713 (Robert Edwards & Stroud joint PI) Dates of Approval (For which this is pertinent)
(Robert Stroud, Leader of Project 2) 08/01/15 – 04/30/19

Vesicular Glutamate transporters. The aims are to elucidate the mechanisms of packaging glutamate, the main neurotransmitter in the brain, into synaptic vesicles by the VGLUT class of transporters. There are fundamental questions about the driving energetics, ions and regulation. We are reporting a high-resolution x-ray structure and functional assays of a bacterial homolog as we pursue CryoEM/X-ray and assays of VGLUTs.

R01 GM024485 (Robert Stroud, PI) Dates of Approval
NIH 03/15/17 – 12/31/20

Molecular Basis for Trans membrane Conduction & Signaling

This project seeks to decode fundamental properties of membrane channels and transporters at the level of atomic structure. One aim concerns secondary transporter conformational dynamics and therapeutic prospectives in the MFS superfamily. I designed research, analysis and wrote the publications.

P01 GM111126 (Stroud, Cheng, Craik, Sali, Dates of Approval
Jacobsen, Kroetz, Tampé) 08/01/15 – 07/31/20

ABC transporter Mechanisms.

(Robert Stroud, Leader of Project 3) The aims are to define the mechanistic cycle of ABC transporters focusing on members of the ABCC and ABCG families, and their importance in drug resistance. Cryo electron microscopy and crystallography are primary tools in the search for conformational states along the mechanistic pathway.

P50 GM0082250 (Nevan Krogan, PI) NIH Dates of Approval
(Robert Stroud, Leader of Project 3) 08/27/07 – 08/31/22

HIV Accessory and Regulatory Complexes

My Project 2 is with Vpu that targets tetherin, and CD4 for destruction. I defined structures for HIV-1 protease with substrates, products, and drug leads, and the first structure of the catalytic and C-terminal domains of HIV-1 integrase. I designed research, analysis and wrote the publications.

Completed Research Support

1U54 GM094625 (Robert Stroud, PI) Dates of Approval
NIH 09/30/2010 – 06/30/15

Center for Structures of Membrane Proteins

5 investigator Investigators devoted to enabling determination of the three dimensional atomic structures of integral membrane proteins of high biomedical impact, and their heteromeric complexes.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Li, Fei

eRA COMMONS USER NAME (credential, e.g., agency login): msuli1

POSITION TITLE: Postdoc scholar

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
Xiamen University	BS	07/2008	Chemistry
Michigan State University, East Lansing, MI	Ph.D	08/2014	Biochemistry and Molecular Biology
Michigan State University, East Lansing, MI	Postdoctoral Fellow	05/2015	Biochemistry and Molecular Biology
The Scripps Research Institute, La Jolla, CA	Postdoctoral Fellow	11/2015	Integrative Structural and Molecular Biology
University of California, San Francisco, San Francisco, CA	Postdoctoral Fellow	present	Biochemistry and Biophysics, Neurology

A. Personal Statement

My long-term research interest concerns the function of important membrane proteins at a molecular level. In particular, I am interested in membrane proteins involved in human disease, where a detailed understanding of structure and function would help to design treatment.

I obtained my Ph.D with Dr. Shelagh Ferguson-Miller, a pioneer in membrane protein biochemistry and mitochondrial function. Working with her on what was then a new project in the lab, the mitochondrial translocator protein 18 kDa (TSPO), I obtained solid training in the expression, purification and characterization of membrane proteins. With the help of Dr. Michael Garavito, I learned techniques of structure determination by X-ray crystallography, especially using the lipidic cubic phase (LCP) method. I chose Drs. Stroud and Edwards as mentors for my postdoctoral training because they together provide a broad research program for me to learn new techniques and to explore new research avenues. With their support, I am obtaining training in multiple aspects of structural biology as well as cell biology and physiology. My current research focuses on structural and functional characterization of vesicular glutamate transporters (VGLUTs).

VGLUTs represent a large family of membrane transporters that are previously inaccessible for structural determination by cryo-EM: Solute Carrier (SLC) proteins mediate transports of various molecules across the membrane and play important roles in normal physiology as well as disease. However, due to their small size, and conformation changes associated with function, structural determination of SLC proteins remain challenging. To facilitate structural determination of VGLUTs by cryo-EM, I have raised high affinity monoclonal antibodies against VGLUTs and obtained promising preliminary structure at resolution around 5 Å. Successful structural determination of VGLUTs will not only provide the first atomic structure of any synaptic vesicle membrane protein and serve as the basis for functional characterization but also demonstrate the feasibility of cryo-EM as a valuable tool in characterizing small and dynamic membrane transporters.

1. Li F, Liu J, Valls L, Hiser C, Ferguson-Miller S. Identification of a key cholesterol binding enhancement motif in translocator protein 18 kDa. **Biochemistry**. 2015 Feb 24;54(7):1441-3. PubMed PMID: [25635829](#); PubMed Central PMCID: [PMC5125615](#).
2. Li F, Liu J, Zheng Y, Garavito RM, Ferguson-Miller S. Protein structure. Crystal structures of translocator protein (TSPO) and mutant mimic of a human polymorphism. **Science**. 2015 Jan 30;347(6221):555-8. PubMed PMID: [25635101](#); PubMed Central PMCID: [PMC5125025](#).

3. **Li F**, Xia Y, Meiler J, Ferguson-Miller S. Characterization and modeling of the oligomeric state and ligand binding behavior of purified translocator protein 18 kDa from *Rhodobacter sphaeroides*. **Biochemistry**. 2013 Aug 27;52(34):5884-99. PubMed PMID: [23952237](#); PubMed Central PMCID: [PMC3756528](#).
4. Smirnova IA, Sjöstrand D, **Li F**, Björck M, Schäfer J, Östbye H, Högbom M, von Ballmoos C, Lander GC, Ädelroth P, Brzezinski P. Isolation of yeast complex IV in native lipid nanodiscs. **Biochim Biophys Acta**. 2016 Dec;1858(12):2984-2992. PubMed PMID: [27620332](#).

B. Positions and Honors

Positions and Employment

2006 - 2008	Undergraduate research scholar, Laboratory of Dr. Yu-fen Zhao, Xiamen University, China
2009 - 2014	Graduate research assistant, Laboratory of Dr. Shelagh Ferguson-Miller, Michigan State University
2014 - 2015	Postdoc scholar, Laboratory of Dr. Shelagh Ferguson-Miller, Michigan State University
2015 - 2015	Postdoc scholar, Laboratory of Dr. Gabriel Lander, The Scripps Research Institute
2016 -	Postdoc scholar, Laboratory of Drs. Robert Stroud and Robert Edwards, University of California, San Francisco

Other Experience and Professional Memberships

2012 -	Member, Biophysical Society
2016 -	Member, American Heart Association

Honors

2004, 2005	National Scholarship for Fundamental Sciences, Xiamen University
2005, 2006	The 1 st class student Fellowship, Xiamen University
2006, 2007	Outstanding student award, Xiamen University
2007	Zhu-Yuan Scholarship, Zhu-Yuan Foundation
2006 - 2008	Yu-Miao undergraduate research grant, Ministry of Education, China
2012	Travel fellowship to attend biophysical society annual meeting
2012	Graduate Research Enhancement Award, Michigan State University
2013	Dissertation Completion Fellowship, Michigan State University
2014	Jack T. Watson outstanding graduate student award, Michigan State University
2015	Committee for Professional Opportunities for Women travel award, Biophysical Society
2015	Young Bioenergeticist Award, Biophysical Society
2015	Best Poster Award, Membrane Protein Structures 2015 Meeting
2017	Life Science Foundation Fellowship (final list, funding not matched), Life Science Foundation
2017 - 2019	Postdoctoral fellowship, American Heart Association
2019 - 2024	NIH K99 Pathway to Independence Award

C. Contribution to Science

1. **Characterization and structural determination of translocator protein 18 kDa (TSPO)**. My graduate research focused on the characterization and structural determination of translocator protein 18 kDa (TSPO) from *Rhodobacter sphaeroides* (RsTSPO). RsTSPO represents a family of membrane proteins located at the outer mitochondrial or bacterial membrane. Previously identified as the peripheral benzodiazepine receptor, TSPO has been actively investigated as a promising target for Alzheimer's disease, cancer, and inflammatory disease. However, the mechanism of drug interaction and TSPO function remain unknown. For my thesis work, I determined the first crystal structure of a translocator protein family protein bound to the putative endogenous ligand protoporphyrin IX. I also characterized the oligomeric state of RsTSPO and its ligand binding properties. Through these characterizations, I identified a motif critical for cholesterol binding. These results provided the structural basis for understanding TSPO function and developing compounds that target the protein. Additionally, I also characterized and

determined the crystal structure of a mutant, RsTSPO-A139T, which mimics the disease-associated polymorphism observed in humans. Comparison of the structures of wild type and the A139T mutant explains the low affinity of the mutant for cholesterol and drugs.

- a. **Li F**, Liu J, Valls L, Hiser C, Ferguson-Miller S. Identification of a key cholesterol binding enhancement motif in translocator protein 18 kDa. **Biochemistry**. 2015 Feb 24;54(7):1441-3. PubMed PMID: [25635829](#); PubMed Central PMCID: [PMC5125615](#).
 - b. **Li F**, Liu J, Zheng Y, Garavito RM, Ferguson-Miller S. Protein structure. Crystal structures of translocator protein (TSPO) and mutant mimic of a human polymorphism. **Science**. 2015 Jan 30;347(6221):555-8. PubMed PMID: [25635101](#); PubMed Central PMCID: [PMC5125025](#).
 - c. **Li F**, Xia Y, Meiler J, Ferguson-Miller S. Characterization and modeling of the oligomeric state and ligand binding behavior of purified translocator protein 18 kDa from *Rhodobacter sphaeroides*. **Biochemistry**. 2013 Aug 27;52(34):5884-99. PubMed PMID: [23952237](#); PubMed Central PMCID: [PMC3756528](#).
2. **Developed a method for *de novo* phasing of membrane protein crystals**. Structural determination of RsTSPO was extremely challenging in several respects. In particular, crystals obtained from the lipidic cubic phase (LCP) environment were extremely small and fragile, making it very difficult to collect high quality diffraction data required for phasing with selenomethionine, or to derivatize with heavy metal through conventional soaking method. Since RsTSPO belong to a novel structural fold, experimental phasing was required. To minimize crystal manipulation and enable high-throughput screening, I developed a modification of the standard LCP method, now called the LCP-HA method that is a convenient semi-cocrystallization method utilizing the same crystal setup for standard LCP crystallization, to screen for heavy metal derivatives in the LCP environment. Compared to traditional soaking and co-crystallization, LCP-HA does not need any crystal manipulation and confers high success rate with the capability of high throughput. This method played a critical role in the successful structure determination of RsTSPO.
- a. **Li F**, Liu J, Zheng Y, Garavito RM, Ferguson-Miller S. Protein structure. Crystal structures of translocator protein (TSPO) and mutant mimic of a human polymorphism. **Science**. 2015 Jan 30;347(6221):555-8. PubMed PMID: [25635101](#); PubMed Central PMCID: [PMC5125025](#).
3. **Characterized membrane proteins extracted with native lipid lipodisqs®**. The structure and function of membrane proteins are heavily influenced by the lipid/detergent environment in which they are extracted. In recent years, alternative solubilization agents have been developed to preserve native lipids during purification. Among these, styrene maleic anhydride (SMA) co-polymer shows unique promising properties: rather than replacing detergent after solubilization, it can be used directly for solubilization, thus avoiding the need for detergent extraction that disrupts the association with native lipids. In additionally, after solubilization in SMA, no excess SMA monomer is needed in solution, avoiding interference by empty detergent micelles with downstream purification and characterization. To better understand how SMA might influence the function and structure of membrane proteins, we used electron microscopy to characterize yeast Complex IV solubilized by SMA in native lipid lipodisqs®.
- a. Smirnova IA, Sjöstrand D, **Li F**, Björck M, Schäfer J, Östbye H, Högbom M, von Ballmoos C, Lander GC, Ädelroth P, Brzezinski P. Isolation of yeast complex IV in native lipid nanodiscs. **Biochim Biophys Acta**. 2016 Dec;1858(12):2984-2992. PubMed PMID: [27620332](#).

Complete List of Published Work in My Bibliography:

<http://www.ncbi.nlm.nih.gov/myncbi/collections/bibliography/50153125/>

D. Research Support

ACTIVE

17POST33660928 (Li)

American Heart Association

The Regulation of Glutamate Release by Protons

Goal: To provide a scientific basis for developing treatments and compounds that block the allosteric activation of VGLUTs to reduce glutamate release and alleviate excitotoxicity.

07/01/2017 – 06/30/2019

\$54,316

10.02 cal mos

Role: PI

PENDING

1K99MH119591 (Li)

07/01/2019 – 06/30/2024

12.0 cal mos

NIH/NIMH

Structural Basis of Quantal Release

Goal: To provide the basis for understanding the molecular function and regulation of vesicular glutamate transports (VGLUTs) and synaptic vesicle function. This grant also provides the training opportunities to facilitate career development as an independent investigator.

Role: PI

Completed

National Fund for Fundamental Sciences, Ministry of Education, China

04/01/06-04/01/08

Synthesis of chiral phosphoryl compounds *via* ester-exchange method

Yu-Miao undergraduate research grant that provides research support for highly motivated undergraduate students to conduct proposed research in a research laboratory for 2 years during their senior years.

Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Robert H. Edwards

eRA COMMONS USER NAME (credential, e.g., agency login): RHEDWARDS

POSITION TITLE: Professor of Neurology and Physiology

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Yale College	B.A.	05/1976	Biology
Johns Hopkins Medical School	M.D.	06/1980	Medicine
Johns Hopkins Hospital (internship)		06/1981	Medicine
UCSF Hospital (residency)		06/1984	Neurology
UCSF (postdoctoral fellowship)		08/1990	Biochemistry & Biophysics

A. Personal Statement

The quantal release of neurotransmitter underlies essentially all information processing in the nervous system, and we wish to understand the mechanisms that mediate and regulate this process. Quantal release depends on the vesicular storage of transmitter and the amount of transmitter stored per vesicle also influences quantal size, the elementary unit of synaptic transmission. Using selection in the parkinsonian toxin MPP⁺, we identified the first vesicular neurotransmitter transporter, suggesting that vesicular monoamine transport can protect against neural degeneration. Using this and other vesicular transporters that we have identified, we now wish to understand the mechanisms that regulate their activity, including the H⁺ electrochemical driving force. To circumvent limitations associated with the functional analysis of transporters on intracellular membranes, we are developing new methods, including electrophysiology, to record from transporters both at the plasma membrane and on endosomes.

We also use the transporters to study the membrane trafficking that orchestrates regulated exocytosis. This involves live imaging of cultured neurons and we have used the vesicle transporters to develop new, powerful reporters for the synaptic vesicle cycle that are now in wide use. In our group, this work has led to some of the first evidence for molecular differences in the composition of synaptic vesicles within the recycling and resting pools. At the same time, we have used the transporters to explore the cell biological basis of transmitter corelease, in particular glutamate release by dopamine neurons, identifying novel pathways to regulated exocytosis. We have also used the vesicular monoamine transporter to elucidate the formation of dense core vesicles that release neural peptides.

Over the years, work in the lab has converged on mechanisms relevant for disease. In addition to the neuroprotective role of the vesicular monoamine transporter, we have studied the presynaptic protein α -synuclein, focusing on its physiological role in neurons. We have a particular interest in how normal function evolves into disease, and have recently used knockout mice to identify the normal function of synuclein.

Our goal is thus to address important, basic questions that remain poorly understood and have the potential to change current thinking about neurotransmission and disease. The lab uses a variety of methods, from molecular biology and biochemistry to cell biology, including live imaging of neurons and chromaffin cells and electrophysiology, in heterologous systems and at the synapse. We also use genetic manipulation *in vivo* to test the physiological and behavioral consequences of mechanisms we identify.

B. Positions and Honors

1990-93, Assistant Professor, Neurology, UCLA; 1993-94, Associate Professor, Neurology and Biological Chemistry, UCLA; 1995-97, Associate Professor, Neurology and Physiology, UCSF; 1997- , Professor, Neurology and Physiology, UCSF

Honors

1990-92, March of Dimes Basil O'Connor Award; 1990-93, Alzheimer's Foundation Faculty Scholar Award; 1993, Established Investigator Award, NARSAD; 1999, Smith Lectureship, National Psychobiology Inst., Israel; 2005, Distinguished Investigator Award, NARSAD; 2007, FC MacIntosh Lectureship, McGill; 2012, Institute of Medicine; 2012, Fellow, American Association for the Advancement of Science; 2015, Elliot Royer Award; 2017, elected National Academy of Sciences

Other Experience and Professional Memberships

1993-96, Advisory Board, Hereditary Disease Foundation; 1993-96, Editorial Board, *Neuron*; 1995-99, Neuropharmacology and Neurochemistry Review Section, NIMH; 1995-, Member, American Neurological Association; 1996-99, Scientific Advisory Board, Tourette's Foundation; 1997- , Member, American Society for Clinical Investigation; 1997-2003, Editorial Board, Journal of Neuroscience; 1998-2005, Scientific Advisory Board, National Parkinson's Foundation; 1999-2003, MDCN-5 Review Section, NIH; 2000-, Member, Dana Foundation; 2001-, Co-Director, UCSF Cell Biology Program; 2012-, McKnight Foundation Brain Disorders Award Committee

C. Contribution to Science

1) Identification of the vesicular transporters for classical neurotransmitters

The release of neurotransmitter by exocytosis depends on storage inside specialized secretory vesicles such as synaptic vesicles. Previous work had identified multiple transport activities on synaptic vesicles and characterized their dependence on the H⁺ electrochemical driving force, but the proteins responsible remained unknown. Using selection in the neurotoxin MPP⁺, we identified the first vesicular neurotransmitter transporter, for monoamines, defining a family that also includes the vesicular acetylcholine transporter. In collaboration with E. Jorgensen, we identified the vesicular transporter for GABA and glycine, which defined yet another family of amino acid transporters. We also identified the first vesicular transporter for glutamate, which defines another family of three isoforms. Importantly, these three families show no sequence similarity. They have provided new insight into the biophysical mechanisms involved in neurotransmitter packaging, and new tools to image the cycling of synaptic vesicles. The vesicular glutamate transporters also provided the first unequivocal markers for glutamatergic synapses, tools widely used by the neuroscience community. Most recently, we have used electrophysiology to characterize the properties of these elusive proteins, identifying novel properties such as allosteric activation of an associated Cl⁻ conductance by H⁺.

Liu, Y., Peter, D., Roghani, A., Schuldiner, S., Prive, G.G., Eisenberg, D., Brecha, N., Edwards, R.H. 1992. A cDNA that suppresses MPP⁺ toxicity encodes a vesicular amine transporter. *Cell* 70, 539-551.

Bellocchio, E. E., Reimer, R. J., Freneau, R. T. J., and Edwards, R. H. 2000. Uptake of glutamate into synaptic vesicles by an inorganic phosphate transporter. *Science* 289, 957-960.

Eriksen, J., Chang, R., McGregor, M., Silm, K., Suzuki, T., and Edwards, R.H. (2016). Protons regulate vesicular glutamate transporters through an allosteric mechanism. *Neuron* 90, 768-780.

Ullman, J. C. *et al.* A mouse model of autism implicates endosome pH in the regulation of presynaptic calcium entry. 2018. *Nat. Commun.* 9, 330 (2018).

2) The function of alpha-synuclein

Mutations in alpha-synuclein cause a dominant form of Parkinson's disease (PD) and the protein accumulates in the brain of essentially all patients with sporadic PD. Taken together, these findings suggest that alpha-synuclein has a causative role in the idiopathic disorder. However, the function of synuclein remains unknown. A small protein that localizes to the nerve terminal, previous work had focused almost exclusively on its aggregation. We have complemented this approach by addressing the behavior and function of synuclein in neurons, generally by live imaging. Our work showed that despite its strong presynaptic localization, this peripheral membrane protein interacts only weakly with presynaptic structures by photobleaching. We also demonstrated that activity results in the dispersion of synuclein from the synapse. We then showed that over-expression of the wild type human protein inhibits synaptic vesicle exocytosis. Since we also found that synuclein can bend and fragment membranes, we have recently tested its role in the properties of individual fusion events. The results show that both endogenous and over-expressed synuclein promote dilation of the

exocytic fusion pore, identifying the first physiological defect in knockout mice. We are now working on the mechanism and physiological role of this activity.

Fortin, D.L., Nemani, V.M., Voglmaier, S.M., Anthony, M.D., Ryan, T.A., Edwards, R.H. 2005. Neural activity controls the synaptic accumulation of α -synuclein. *J. Neurosci.* 25, 10913-10921.

Nemani, V.M., Lu, W., Berge, V., Nakamura, K., Onoa, B., Lee, M.K., Chaudhry, F.A., Nicoll, R.A. and Edwards, R.H. 2010. Increased expression of α -synuclein reduces neurotransmitter release by inhibiting synaptic vesicle reclustering after endocytosis. *Neuron* 65, 66-79.

Nakamura, K., Nemani, V.M., Azarbal, F., Skibinski, G., Levy, J.M., Egami, K., Munishkina, L., Zhang, J., Gardner, B., Wakabayashi, J. et al. 2011. Direct membrane association drives mitochondrial fission by the Parkinson Disease-associated protein α -synuclein. *J. Biol. Chem.* (Paper of the Week and among Best of JBC 2011), 286, 20710-20726.

Logan, T., Bendor, J., Toupin, C., Thorn, K. and Edwards, R.H. 2017. α -Synuclein promotes dilation of the exocytotic fusion pore. *Nat. Neurosci.* 20, 681-9.

3) The corelease of classical neurotransmitters

Previous work in culture had suggested that monoamine neurons such as dopamine and serotonin neurons might corelease glutamate, but this was not considered seriously or tested until identification of the vesicular glutamate transporters (VGLUTs). It then became possible to demonstrate the expression of VGLUTs by monoamine neurons *in vivo*. We also tested this possibility by inactivating VGLUT2 specifically in dopamine neurons. This work demonstrated that glutamate helps to make the pH gradient that drives monoamine uptake. In subsequent experiments, we used optogenetics to demonstrate that the glutamate released by dopamine neurons can act as an independent, excitatory signal in the striatum. We also identified an adjacent population of midbrain neurons that releases only glutamate, not dopamine, and inactivation of the VGLUTs has indeed helped to identify a large number of neurons not previously recognized as glutamatergic. Initially considered an unusual property of specific neuronal populations, corelease has now been recognized as a much more widespread phenomenon, although the physiological and behavioral roles have generally remained unknown. Although the observed synergy in storage indicates that at least some vesicles must contain both transmitters, our own work and that of others has also raised the possibility of release from different vesicles and we are interested in the cell biological basis for this phenomenon, which is the basis for this application..

Hnasko, T.S., Chuhma, N., Zhang, H., Goh, G.A., Sulzer, D., Palmiter, R.D., Rayport, S. and Edwards, R.H. 2010. Vesicular glutamate transport promotes dopamine storage and glutamate corelease *in vivo*. *Neuron* 65, 643-656.

Stuber, G.D., Hnasko, T.S., Britt, J.P., Edwards, R.H., and Bonci, A. 2010. Dopaminergic terminals in the nucleus accumbens but not the dorsal striatum corelease glutamate. *J. Neurosci.* 30, 8229-8233.

Hnasko, T.S., Hjelmsted, G.O., Fields, H.L., Edwards, R.H. 2012. Ventral tegmental area glutamate neurons: electrophysiological properties and projections. *J. Neurosci.* 32, 15076-85.

Onoa, B., Li, H., Gagnon-Bartsch, J.A., Elias, L.A., and Edwards, R.H. 2010. Vesicular monoamine and glutamate transporters select distinct synaptic vesicle recycling pathways. *J. Neurosci.* 30, 7917-7927.

4) The biogenesis of large dense core vesicles

Previous work on the biogenesis of large dense core vesicles (LDCVs) has focused primarily on luminal proteins of the dense core which aggregate under the conditions of the trans-Golgi network, where LDCVs form. Essentially nothing has been known about the cytosolic machinery that is involved in LDCV formation. Taking advantage of the vesicular monoamine transporter (VMAT), which localizes preferentially to LDCVs in a number of cell types, we found that its localization to LDCVs depends on a cytosolic sorting sequence. The entire dileucine-like sorting motif promotes multiple trafficking events in the cell, but particular residues upstream of the dileucine are required specifically for sorting to LDCVs. Replacement of these upstream acidic residues increases cell surface expression of VMAT by diverting the transporter from the regulated secretory pathway (LDCVs) to constitutive. We then used the wild type protein to identify mutations in the cell that would phenocopy the effect of trafficking mutations in the transporter, screening *Drosophila* S2 cells by flow cytometry and RNAi directed against all genes conserved to mammals. This screen identified multiple subunits of the adaptor protein AP-3, and subsequent work in mammalian cells confirmed its role in LDCV formation. We then showed that a previously identified AP-3 interacting protein of mysterious function serves

as a novel coat protein for AP-3 in this process: the two genes have the same phenotype, and no additive effect on LDCV function or morphology. This provides some of the first information about cytosolic machinery involved in the formation of LDCVs.

Krantz, D.E., Waites, C., Oorschot, V., Liu, Y., Wilson, R.I., Tan, P.K., Klumperman, J., Edwards, R.H. 2000. A phosphorylation site in the vesicular acetylcholine transporter regulates sorting to secretory vesicles. *J. Cell Biol.* 149, 379-395.

Li, H., Waites, C.L., Staal, R.G., Dobryy, Y., Park, J., Sulzer, D.L. Edwards, R.H. 2005. Sorting of vesicular monoamine transporter 2 to the regulated secretory pathway confers the somatodendritic exocytosis of monoamines. *Neuron* 48, 619-633.

Asensio, C., Sirkis, D., Edwards, R.H. 2010. RNAi screen identifies a role for adaptor protein 3 in sorting to the regulated secretory pathway. *J. Cell Biol.* 191, 1173-1187.

Asensio, C.S., Sirkis, D.W., Maas, J., Egami, K., To, T.-L., Brodsky, F.M., Shu, X., Cheng, Y., Edwards, R.H. (2013) Self-assembly of VPS41 promotes sorting required for biogenesis of the regulated secretory pathway. *Dev. Cell.* 27, 425-437.

5) Identification of the transporters responsible for the glutamine-glutamate cycle

In contrast to most classical transmitters that recycle by uptake across the plasma membrane of the cell that has released them, glutamate recycles through glia. Glial transporters take up the synaptically released glutamate and convert it to glutamine, which then recycles to the glutamatergic terminal. However, the transporters responsible for recycling glutamate back to neurons were not previously known. In the course of studying an orphan transporter related to the vesicular GABA transporter, we identified its substrate by monitoring the movement of the presumed, coupled ion (H^+). We thus identified a transporter that couples the uptake of glutamine and Na^+ in exchange for H^+ , which corresponds to a classical transport system first identified in the liver known as system N. We and others then identified a series of closely related proteins as additional system N and system A isoforms. The system A proteins also transport glutamine, but in an electrogenic manner that would concentrate glutamine more strongly than system N. This work predicted a role for system N transporters in the efflux of glutamine from astrocytes and system A transporters in glutamine uptake by neurons. Recent data has generally corroborated this model, although genetic manipulation enabled by the identification of these proteins should provide a better test. Interestingly, these same transporters appear to have a parallel role in nitrogen metabolism by the liver, which also involves glutamine transport between different populations of cells.

Chaudhry, F.A., Reimer, R.J., Krizaj, D., Barber, D., Storm-Mathisen, J., Copenhagen, D.R., Edwards, R.H. 1999. Analysis of an orphan neurotransmitter transporter identifies novel physiological roles for classical amino acid transport System N in nitrogen metabolism and synaptic transmission. *Cell* 99, 769-780.

Chaudhry, F.A., Schmitz, D., Reimer, R.J., Larsson, P., Gray, A.T., Nicoll, R., Kavanaugh, M., Edwards, R.H. 2002. Glutamine uptake by neurons: interaction of protons with system A transporters. *J. Neurosci.* 22, 62-72.

Chaudhry, F.A., Krizaj, D., Larsson, P., Reimer, R.J., Wreden, C., Storm-Mathisen, J., Copenhagen, D., Kavanaugh, M., Edwards, R.H. 2001. Coupled and uncoupled proton movement by amino acid transport system N. *EMBO J.* 20, 7041-7051.

<http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/41249271/?sort=date&direction=descending>

D. Research Support

CURRENT

R37 MH50712 (Edwards, PI) 7/1/93-7/31/21 3.00 cal. mos.

NIMH

The Transport of Neurotransmitters into Synaptic Vesicles

This project focuses on the mechanism and regulation of vesicular glutamate transport, particularly with regard to its role in synaptic transmission. This grant is complementary to the current application. No overlap.

R01 (Stroud, Edwards co-Pis) 11/1/14 – 10/31/19 1.8 cal. mos.

NINDS

The Structural Basis for Vesicular Neurotransmitter Transport

This joint project addresses the structural basis of vesicular glutamate transporters and their relatives. The current application seeks to renew this grant.

R01 NS103938 (Edwards, PI) 9/1/17–8/31/22 3.0 cal. mos.

NINDS

Neurotransmitter Corelease

This project examines the molecular and cellular basis of neurotransmitter corelease. No overlap..